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Identification and characterization of a new virus in the genus *Potyvirus* from wild populations of *Angelica lucida* L. and *A. genuflexa* Nutt., family *Apiaceae*

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Summary

A novel potyvirus was discovered in *Angelica lucida* L. (wild celery) and *A. genuflexa* Nutt. (kneeling Angelica) (family *Apiaceae*) in the Matanuska-Susitna Valley, Alaska. The experimental plant host range of the virus included species in three families: *Chenopodiaceae* (*Chenopodium amaranticolor* Coste et Reyn and *Chenopodium quinoa* Willd.), *Solanaceae* (*Nicotiana benthamiana* Domin, and *N. clevlandii* Gray.), and *Apiaceae* (*Anethum graveolens* L., *Apium graveolens* L. var. *dulce* (Miller)), *Daucus carota* L. subspecies *sativus* (Hoffm.) Arcang., and *Petroselinum crispum* (Miller) Nyman ex A. W. Hilland). The virus contained flexuous rods with an ssRNA genome ∼9.5–10 k nts and a CP (∼35 kDa) that reacted to a universal potyvirus monoclonal antibody in Western blot analysis. The sequenced genomic 3′-end (∼1850 nt) contained a potyvirus genomic arrangement that included the 3′-terminus of the NIb (nuclear inclusion) gene, the CP (coat protein) gene, and a 3′-UTR (untranslated region) attached to a poly(A)tail. The CP amino acids had between 54 and 70% identity with 12 selected members from the genus *Potyvirus.* Phylogenetically, the Alaskan potyvirus clustered with three other apiaceous potyviruses from Australia. The novel Alaskan virus was confined to *A. lucida* L. and *A. genuflexa* Nutt. in nature, and was classified in the genus *Potyvirus*, family *Potyviridae*, and in part named after its natural plant hosts, angelica virus Y (AnVY).

Introduction

Native plant species that contribute to the natural landscape in remote regions of Alaska are also represented in remnant areas intermixed with agricultural and residential lands in the Matanuska–Susitna Valley of south central Alaska. They grow along the roadside among invasive weeds, in residential gardens, on the edge of crops, and on undeveloped wooded lots. Like most regions throughout the world, plant viral disease studies in Alaska have been mainly confined to economically important crops, with few observations and surveys of wild

Note: Nucleotide sequence data reported are available in the GenBank databases under accession numbers: EF488740, EF488741.

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plants growing in their natural environments [4, 6, 7, 13, 14, 17, 20, 23]. A novel carmovirus confined to wild *Lupinus nootkatensis* Donn ex Simm near the headwaters of the Little Susitna River in the Talkeetna Mountains [19], and a unique carlavirus and potyvirus complex that is distributed throughout south central Alaska in twisted stalk, *Streptopus amplexifolius* (L.) DC. [20], are examples of two recent efforts to expose the existence of plant viruses in Alaskan natural landscapes.

In 2004, a wild celery plant, *Angelica lucida* L., growing on the edge of a road in Palmer, Alaska, had distinctly mottled leaves that resembled viral symptoms. Western blots of protein extracts from partially purified virus particles derived from the leaves of the diseased wild celery plant suggested that the plant was infected by a potyvirus. The next year, the same plant and several other *A. lucida* L. plants appeared with similar symptoms and also were potyvirus positive by Western blot.

Species in the genus *Angelica* are classified in the carrot family *Apiaceae* (formerly *Umbelliferae*) and are mostly represented in the northern hemisphere. They are biennials or perennials with deep tap roots and contain diagnostic umbrellalike compound inflorescences called umbels [8, 16]. The two native species in Alaska, *A. lucida* L. and *A. genuflexa* Nutt. (kneeling Angelica), inhabit moist environments along streambanks, with the former growing on beaches and inland meadows, and the later in moist thickets, forest openings and edges [24]. *Angelica lucida* L. is widely distributed along the coastal regions and in the Alaskan Range, and *A. genuflexa* Nutt. occurs from the Aleutians to the coastal zone of southeastern through central Alaska [8, 24].

In 2005–06, *A. lucida* L. and *A. genuflexa* were surveyed in south central Alaska for viruses, with an emphasis on identifying the tentative potyvirus and studying its biology. The potyvirus was named in part after its natural host and will be referred to as angelica virus y (AnVY).

Materials and methods

Virus source

In 2005–06, individual plants of *A. lucida* L. were surveyed on ~5 km of roadsides in a residential/farming community (61°37′N, 149°13′W) near Palmer, Alaska. Leaves from plants with and without symptoms were collected and processed within a week or stored at −80 °C. Similar collections from *A. genuflexa* Nutt. plants took place at a remote site (61°57′N, 150°58′W) near Skwentna. These plants provided the virus for characterization and transmission studies discussed below.

Virion extraction and characterization

Virions were extracted from leaf tissue and partially purified as described by Lane [10, 11]. Collection and detection assays used 1 g leaf tissue/plant that was homogenized with 20 ml 0.4 M sodium citrate buffer (pH 6.7) containing 0.15 ml of 0.5 M sodium diethydithiocarbamate; the protocol was also scaled up with ~25 g tissue/500 ml buffer to obtain greater virion concentrations. The sap was expressed through muslin and centrifuged for 10 min at 111,000 *g* in a Beckman 50.2 Ti rotor. The supernatant was filtered through Miracloth (CalBiochem, La Jolla. CA, USA), and 0.3 ml 10% Triton X-100 was added before another centrifugation at 111,000 *g* for 45 min. The final virus pellet in each tube was resuspended in 100–150 µl sterile water and stored at −80 °C.

Formvar-carbon coated grids (Ted Pella Inc., Redding, CA) were placed on drops of the partially purified particles and stained with 2% uranyl acetate. The virus particles were examined and digitally photographed with a JEM-1200EX electron microscope (JEOL USA Inc., Peabody, MA).

The virion RNA was removed from partially purified virus samples as previously described [18] and visualized on ethidium-bromide-stained non-denaturing 1% agarose gels with RNA markers (Invitrogen, Life Technologies, Carlsbad, CA, USA).

Protein extracts from the virion preparations were processed with Precision Plus Protein™ Standards (Bio-Rad, Hercules, CA, USA) and visualized on 12% SDS-PAGE gels with Coomassie Blue R-250 [9] or by Western blots. The separated proteins were blotted onto nitrocellulose membranes and screened for potyvirus with universal potyvirus antiserum (Agdia Inc., Elkhart, Indiana, USA) diluted 1:2000 and goat anti-mouse Ig-G-conjugated alkaline phosphatase (1:3000) using the protocol for the Immuno-Blot Colorimetric assay kit (Bio-Rad, Hercules, CA, USA).

Natural plant host range

Native plant species, *A. lucida* L., *Heracleum lanatum* Michx. (cow parsnip), and *Cicuta mackenzieana* Raup (Mackenzie water hemlock), were surveyed from disturbed ecosystems near agricultural and residential lands on the Palmer site (61°37′N, 149°13′W) in 2005 and/or 06. While the majority of angelica and cow parsnip were sampled along the roadside, the water hemlock samples were collected in a marsh next to Walby Lake. On the Skwentna site, *A. genuflexa* Nutt. plants were examined from a remote natural ecosystem

(61°57′N, 150°58′W) that was not accessible by automobile. Leaves were also collected in 2006 from two other native *Apiaceae* species, *Ligusticum scoticum* L. and *Cnidium cnidiifolium* (Turcz.) Schischk. that were grown and maintained at the Plant Materials Center (State of Alaska, Department of Natural Resources) about 18 km from the Palmer site; the seed sources for *L. scoticum* and *C. cnidiifolium* were from Casco Bay in the Aleutian Islands and Delta Junction, Alaska, respectively.

Experimental plant host range

Partially purified AnVY virions from naturally infected *A. lucida* L. were applied with a pipette to Celite® dusted leaves of test plants, and gently rubbed with a cotton swab or gloved fingers (~10 µl/plant). Viral indicator plants included: C*henopodium amaranticolor* Coste et Reyn., *C. quinoa* Willd., *Nicotiana benthamiana* Domin, and *N. clevlandii* Gray. Selected cultivated species of *Apiaceae* were similarly inoculated with AnVY that was maintained and propagated in *N. benthamiana*. Seed for the following accessions was obtained from the seed bank in the National Plant Germplasm System, United States Department of Agriculture: dill (*Anethum graveolens* L.), celery (*Apium graveolens* L. var. *dulce* (Miller)), carrot, (*Daucus carota* L. subspecies *sativus* (Hoffm.) Arcang.), and parsley (*Petroselinum crispum* (Miller) Nyman ex A. W. Hilland) were inoculated at the seedling stage. All inoculated plants were observed for symptom development and assayed for potyvirus and AnVY detection by Western blot and RT-PCR. The plants were placed in growth chambers from two to at least five months with temperature/light cycles set at 16 h light, 22 °C and 8 h dark, 20 °C.

Reverse transcriptase-polymerase chain reaction, cloning, genomic sequencing, and analysis

All RT-PCR assays used total RNA extracts from 50 to 100 mg leaf tissue using the RNeasy® plant mini kit (QIAGEN Inc., Valencia, California, USA) as directed and the total RNA was stored at −80 °C. Leaves from two naturally infected native plants, *A. lucida* L. (Palmer site) and *A. genuflexa* Nutt. (Skwentna site), were collected in July 2005 and August 2006, respectively. Their total RNA preparations were used in an RT-PCR protocol that specifically targeted potyviruses and spanned the 3′-terminus of the genome that included the 3′-part of the NIb gene, the coat protein (CP) gene, and the 3′-untranslated region with an attached poly(A) tail [3]. The predicted PCR fragments were excised from agarose gels, cleaned with a QIAquick gel extraction kit (QIAGEN Inc.), and cloned into pGEM-Teasy vector (Promega, Madison, Wisconsin, USA) following the manufacturer's instructions. Plasmids were introduced by transformation into *Escherichia coli* XL-2 Blue (Statagene, La Jolla, CA, USA) and isolated with a Qiaprep spin miniprep kit as directed (Qiagen Inc.). The cloned viral inserts were sequenced on both strands by Davis Sequencing Inc. (Davis, CA, USA).

Selected internal sequences derived from the virus-infected *A. lucida* (2005) were then used to design the following primers to specifically detect AnVY in subsequent RT-PCR assays: Angel-F1 (5′-GAACAAGCACCATA CAACG-3′), Angel-F3 (′5-TCCACAAAACCAGAGCA TCG-3′), Angel-R2 (′5-AGCAGGCGAGACTTCGGTC-3′), and Angel-F5 (′5-TTACAGGAAATTCCCGCTGC-3′). The predicted fragments lengths were: Angel-F1/-R2 = 798 nt, Angel-F3/-R2 = 906 nt, and Angel-F1/-R5 = 641 nt. The temperature/time cycle was set at: 1 min 94 °C, followed by 35 cycles of 1 min 94 °C, 2 min 56 °C, and 3 min 72 °C, and ended with 10 min 72 °C. All transmission experiments and 2006 surveys incorporated RT-PCR assays for AnVY detection with Angel-F3/-R2 and/or Angel-F1/-R5 primer sets. The PCR fragments were processed with the QiaQuick PCR purification kit (Qiagen Inc.) before direct sequencing on both strands with the appropriate primers by Davis Sequencing, Inc.

The program ClustalX [22] was used to align selected sequences and generate a phylogenetic tree by the neighbor-joining (NJ) method and bootstraps analysis of 1000 replicas.

Results

Natural plant host range

In 2005, ten *A. lucida* L. plants were observed and leaves sampled between June and August on the Palmer site. No symptoms to very faint mottling were observed on plants in early June, but increased leaf mottling, vein-clearing, and plant stunting appeared several weeks later; all plants tested positive for AnVY. In 2006, a similar pattern occurred with mottled leaves (Fig. 1a) except by July more plants were stunted with severe symptoms that included curled and rugose leaves, with 12 out of 18 plants infected. However, based on Western blots of virus protein extracts from minipurification of collected leaf tissue, the viral titre greatly decreased in older plants, and in some of the same infected plants, no virus was detected. Isolates from *A. lucida* L. were identified as AnVY-l.

Two other species of *Apiaceae* on the Palmer site, *H. lanatum* Michx. (cow parsnip) plants with distinct vein-clearing and mosaic, and *C. mackenzieana* Raup (Mackenzie water hemlock) plants with no symptoms, growing in a marshy area on the edge of a lake near diseased *A. lucida* L. plants,

Fig. 1. Symptoms on naturally (**a**, **b**) and experimentally (**c**, **d**) AnVY (angelica virus y)-infected plants: (**a**) *Angelica lucida* L. and (**b**) *Angelica genuflexa* Nutt. with mottled leaves, (**c**) *Nicotiana benthamiana* with curled and mottled leaves, and (**d**) dill (*Anethum graveolens* L.) with wilted, necrotic dead plants (left) and healthy non-inoculated AnVY plants (right)

did not contain detectable AnVY in the 2005–06 surveys.

In July 2006, on the Skwentna site, *A. genuflexa* plants had mottled leaves (Fig. 1b) that contained flexuous rods with a CP of ~35 kDa and tested positive for potyvirus by Western blot. Extracted total RNA generated the predicted PCR fragments with the universal potyvirus- and AnVY-specific primers, which in turn contained similar sequences to those derived from *A. lucida* from the Palmer site. Isolates from *A. genuflexa* were named AnVY-g.

The last two *Apiaceae* species surveyed in 2006 (October), *L. scoticum* L. and *C. cnidiifolium* (Turcz.) Schischk. on the PMC site, did not have noticeable symptoms or detectable AnVY by RT-PCR assays or minipurifications/protein extractions.

Transmission and experimental plant host range

Virus from partially purified preparations or leaf extracts from naturally infected *A. lucida* L. plants was successfully transmitted to *C. amaranticolor* Coste et Reyn, *C. quinoa* Willd., and *N. benthamiana* Domin.; the first two had local lesions on inoculated leaves, and the third showed stunting and systemic leaf mottling in young plants (Fig. 1c) and curled, thickened rugose leaves in older plants. *Nicotiana benthamiana* Domin. was used as an effective maintenance and propagation host for AnVY; sap and virion extracts were monitored by Western blots and/or RT-PCR. *Nicotiana clevlandii* Gray was a nonsymptomatic host to AnVY when inoculated with extracts from *N. benthamiana* but not from the natural host *A. lucida* L.

Table 1. Mechanical transmission assays of Angelica virus Y (AnVY) to four plant species in the family *Apiaceae*

Plant species[a] (Accession no.)	Symptoms[b]		*RT-PCR[c]		*RT-PCR[d]
Anethum graveolens L. (dill)					
AMES 1666	w, n, d		11/11		N/A
AMES 7787	w, n, d		9/9		N/A
AMES 19087	w, n, d		2/2		N/A
AMES 19179	w, n, d		N/A		3/5
NSL 6417	w, n, d		10/10		N/A
PI 305462	w, n, d		N/A		5/5
PI 307648	w, n, d		N/A		9/9
PI 344265	w, n, d		N/A		1/1
PI 506433	w, n, d		7/7		8/8
		Total	46/46	+	26/28 = 72/74 (97%)
Apium graveolens var. *dulce* (Mill.) Pers. (celery)					
PI 176870	lc-ns		0/10		6/6
PI 222967	ns		1/2		6/9
PI 357327	lc, m, ns		4/4		10/11
PI 379083	rm-ns		7/10		N/A
PI 385950	m-ns		N/A		4/7
		Total	12/26	+	26/33 = 38/59 (64%)
Daucus carota L. subsp. *sativus* (Hoffm.) Arcang. (carrot)					
AMES 19238	ns		1/6		N/A
AMES 25062	ns		4/9		N/A
AMES 25568	ns		2/2		N/A
NSL 6780	ns		4/8		N/A
PI 502239	ns		N/A		0/4
PI 502920	ns		N/A		1/2
PI 632382	ns		N/A		1/6
PI 632387	ns		N/A		9/11
		Total	11/25	+	11/23 = 22/48 (46%)
Petroselinum crispum (Mill.) Nyman ex A. W. Hill (parsley)					
AMES 4545	ns		10/12		N/A
AMES 13817	ns		2/4		N/A
AMES 13819	lc-ns		0/3		7/7
NSL 92006	n-ns		0/1		6/7
PI 140941	m		N/A		2/3
PI 173009	m-ns		N/A		4/4
		Total	12/20	+	19/21 = 31/41 (76%)

[a]Plant species accessions from GRIN (Germplasm Resource Information Network).
[b]Symptoms are denoted as: *d* Death, *lc* leaf curl, *m* mottle, *n* necrosis, *ns* no symptoms, *rm* rugose mosaic, and *w* wilt. The inocula (virions) applied to the plants were extracted from AnVY-infected.
[c]*Angelica lucida* Michaux or AnVY-infected.
[d]*Nicotiana benthamiana* Domin.
*Confirmation of infection was based on RT-PCR assays with AnVY-specific primers that generated either 906-bp or 640-bp fragments; the numerator = number of infected plants, and denominator = total number of inoculated plants, and N/A = not applicable.

Dill, carrot, celery, and parsley seedlings were all susceptible to AnVY, with the greatest incidence of infection in dill and celery (Table 1). The effect AnVY had on seedlings varied from no apparent symptoms, on all carrots and most parsley, to distinct mottling in several celery accessions, to wilt-

ing, necrosis and death in nearly all the dill (Fig. 1d). Older infected celery plants developed curled, rough and thickened leaves, and several parsley plants had distinct mottling on their older leaves; the previously mottled celery plants contained additional prominent vein-clearing on their older leaves. A direct correlation between strong symptom severity with increased PCR product concentration was observed in all the cultivars of young plants (1–4 weeks), followed by a decreased PCR product concentration in aged infected plants (6–30 weeks).

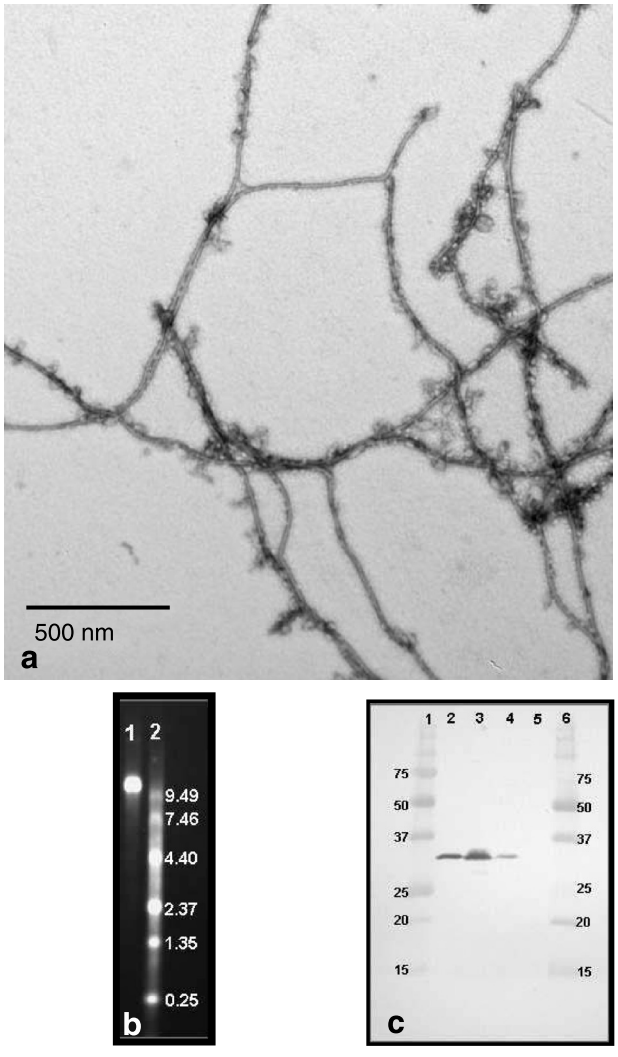


Fig. 2. Characteristics of partially purified angelica virus Y preparations. (**a**) Electron micrograph of virus particles from infected *Nicotiana benthamiana.* (**b**) Non-denaturing agarose (1%) gel of virion RNA (*1*) and RNA markers (*2*). (**c**) Western blot of coat protein from infected *N. benthamiana* (*2*), infected *Angelica lucida* (*3*), infected *A. genuflexa* (*4*), and healthy plant sap (*5*) probed with universal potyvirus antiserum; molecular marker weights, kDa (*1*, *6*)

Virion characterization

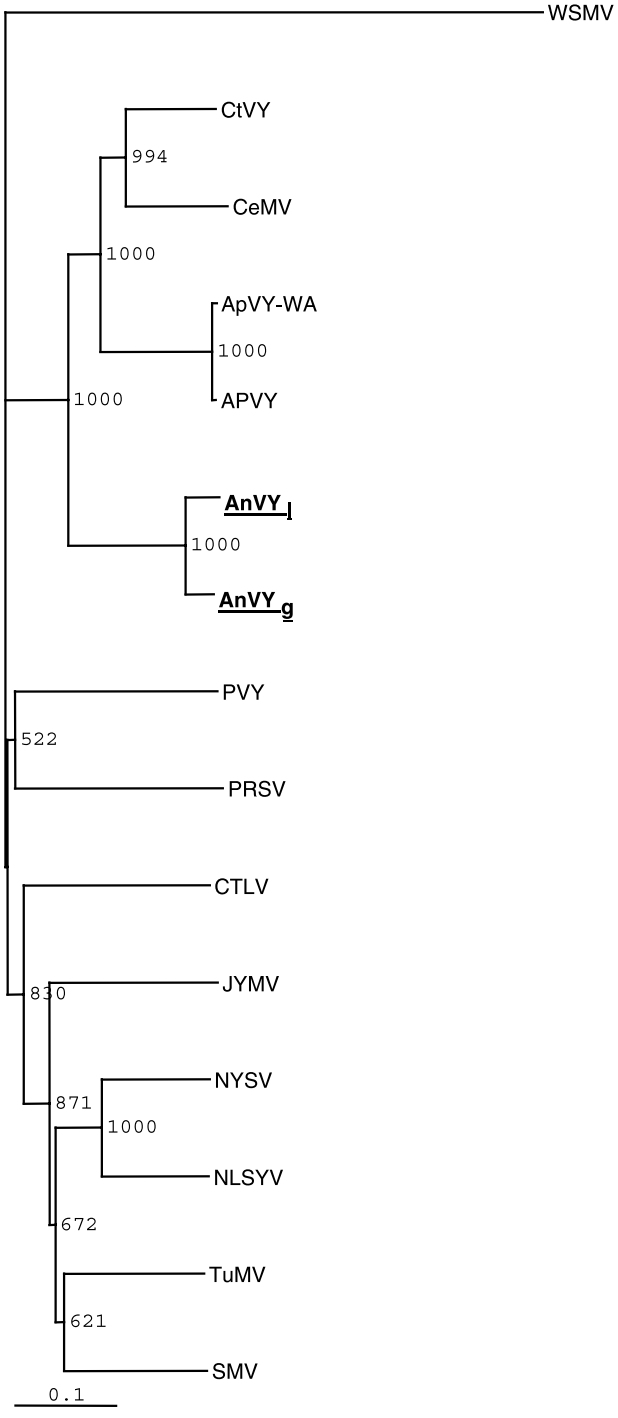
Partially purified virus particles from *A. lucida*, *A. genuflexua*, and inoculated *N. benthamiana* plants were flexuous rods with diameters of ∼12 nm.; the exact lengths were not determined since most of the particles were either attached together and/or broken (Fig. 2a). The genomic single-stranded RNA size extracted from wild *A. lucida* plants was between 9.5 and 10 k nts (Fig. 2b). A single virion CP of ∼35 kDa was detected on Coomassie Blue R-250-stained gels and by Western analysis with universal potyvirus antiserum (Fig. 2c).

PCR and genomic 3′-termini sequences

The viral genomic 3′-termini of two isolates derived from *A. genuflexa* and *A. lucida*, were sequenced from cloned RT-PCR fragments (∼1.8 kbp) and entered in GenBank as: Angelica virus Y-genuflexa, AnVY-g (EF488741) and Angelica virus Y-lucida, AnVY-l (EF488740). AnVY-g (1850 nt) and AnVY-l (1852) were 90 and 94% identical in their nucleotide and amino acid sequences, respectively. Represented genes from both isolates included: 3′-end of the NIb (polymerase) gene (1–519 nt), CP gene (520–1464 nt); the 3′ UTR, AnVY-g (1465–1850 nt) and AnVY-l (1465–1852 nt) attached to a poly(A) tail. The protein motif, GDD, associated with RdRp, was detected in the NIb gene(102–120 nt). Homologues of the motif for all aphid-transmitted potyviruses in the N-end of the CP [12] were detected for AnVY-g as RDIDAG (754–771 nt) and for AnVY-l as RDINAG (754–771 nt).

When compared to other viruses in GenBank with BLASTn,-x, significant matches to sections of AnVY-g,-l sequences were restricted to only species in the family *Potyviridae*, genus *Potyvirus*, and none were close enough (CP amino acids $<80\%$) to belong to the same species as AnVY. Twelve selected potyvirus members were then compared with 487 amino acids from N1b/CP of AnVY-g and AnVY-l, with identities ranging between 57 and

71%. The CP amino acids identities were similar, with 92% identity between the two AnVY isolates, and from 54 to 70% with the other 12 potyviruses. The phylogenetic tree (Fig. 3) grouped AnVY-g and AnVY-l together and formed a cluster with members of four other potyvirus species, carrot virus y (CtVY), celery mosaic virus (CeMV), apium virus y strain *conium maculatum* (ApVY-c), and the parsley isolate ApVY-WA, which infects cultivated, feral, and native *Apiaceae* plants in Australia. When comparing the nucleotide sequences from the NIb/CP genes together, and the 3′-UTR separately, AnVY-g and AnVY-l had 89 and 94% identity (respectively) with each other, and 66–72 and 33–35% (respectively) with the other four viruses in the same cluster.



The PCR assays using primer sets that were designed from the internal sequences of AnVY-l successfully detected the predicted fragment sizes (not shown); the Angel-R2/-F3 primers (PCR product = 906 bp) and/or Angel-R1/F5 (PCR fragment = 641 bp) were selected to be used in the PCR AnVY detection assays for the natural and experimental plant host range previously described. Selected PCR fragments from each of the experimental plant hosts were directly sequenced to definitely confirm virus identification, resulting in nearly identical nucleotide matches with the AnVY-l isolate.

Discussion

This study revealed a new plant virus that had characteristics similar to members of viral species in the

Fig. 3. Phylogenetic tree showing the relationship of two Angelica virus Y (AnVY) isolates with selected members in the genus *Potyvirus*, family *Potyviridae*, based on amino acid alignments of the coat protein and 3′-end of the NIb genes. Depicted virus (Acronym, Accession Number): Angelica virus Y-genuflexa (AnVY-g, EF488741), AnVY-lucida (AnVY-l, EF488740), Apium Virus Y-parsley isolate (ApVY-WA, AF207594), Apium Virus Y-conium maculatum isolate (ApVY, AY049716), celery mosaic virus (CeMV, AF203531), carrot virus Y (CtVY, AF203538), carrot thin leaf virus (CTLV, AF203530), Japanese yellow mosaic virus (JYMV, AB016500), potato virus Y (PVY, AJ439545), papaya ringspot virus (PRSV, AY162218), Narcissus late season yellows virus (NLSYV, AJ493579), Narcissus yellow stripe virus (NYSV, AM158908), scallion mosaic virus (ScMV, AJ316084), turnip mosaic virus (TuMV, AB093602), and as the outgroup (genus *Tritimovirus*, wheat streak mosaic virus (WSMV, AF057533). The numbers at the node indicate values greater than 50% with a bootstrap analysis of 1000 replicates

family *Potyviridae*, genus *Potyvirus*. The viral particles were flexuous rods that consisted of a ssRNA genome, ~9.5–10 k nts, enclosed by a single CP species ~35 kDa. Western blots confirmed potyvirus identity by positive reactions to a universal ''potyvirus'' monoclonal antibody. Definitive potyvirus identification was obtained from genetic analysis of the 3′-end of the genome, which included ~1850 nt attached to a poly(A) tail. The CP amino acid sequence identity when comparing AnVY with known potyvirus meets the criteria for inclusion in the genus *Potyvirus* (40–70%) and its identification as a member of a distinct species (<80%) within the genus *Potyvirus* [2]. The two sequenced isolates from the Skwentna and Palmer sites, AnVY-g and AnVY-l, respectively, may represent strains due to their high nucleotide and amino acid identities. Expanded plant host range studies for AnVY-g and AnVY-l may provide a differential host range between the two isolates. Based on a recent study by Adams et al. [1], the sequences obtained from the cylindrical inclusion (CI) gene on the 5′-half of the genome provided a better representation for the entire genome when compared with the CP.

Like many potyviruses, AnVY-g and AnVY-l were also easily mechanically transmitted to other plant species. Since the natural hosts of AnVY were confined to two plant species in the family *Apiaceae*, and the experimental plant host range consisted of species in *Chenopodiaceae* and *Solanaceae* known for their lack of resistance for many viruses, it was not surprising to find more susceptible species in *Apiaceae*. It would be interesting to test for susceptible plants in other families to determine if AnVY has a wider host range. The selected *Apiaceae* host range species were similar to the cultivated crops grown in the Matanuska-Susitna Valley, where recurring AnVY-infected *A. lucida* plants were found. Preliminary surveys in carrot crops in late September 2006 did not find any virus infections including AnVY.

The distribution of naturally infected *A. lucida* plants was scattered and in clusters, suggestive of an insect vector and/or mechanical transmission. On the Skwentna site (~150 km NW of the Palmer site), five AnvY-g-infected *A. genuflexa* plants out of 12 were not evenly distributed, indicative of an insect vector. The vector is likely an aphid species since AnVY-g and AnVY-l contained the amino acid motif that is characteristic of most aphid-transmitting potyviruses. Preliminary data from seedlings derived from seed of AgVY-l-infected *N. benthamiana* suggest that AnVY is not seed-transmitted. Seed transmission studies that incorporate seed from naturally infected *Angelica* sp. plants would help explain the distribution of diseased plants in nature.

AnVY clustered with several other viruses that infect the same botanical family, *Apiaceae*. Previous to this study, wild umbelliferous plants found in surveys to have viruses include *Conium maculatum* (poison hemlock) in California [21]; *Angelica sylvestris*, *Daucus carota* (carrot), *Anthriscus sylvestris* (cow parsley), *Heracleum sphondylium* (cow parsnip), and *Pastinaca sativa* (parsnip) in the Netherlands [23]; and *Apium prostratum* (sea celery), *D. carota*, and *C. maculatum* in Australia [15]. AnVY was found in only two plant species, *A. genuflexa* and *A. lucida*, each from a geographically distinct site about 150 km apart. AnVY was not detected in two other apiaceous plant species (*H. lanatum* Michx., and *C. mackenzieana* Raup) growing near infected *A. lucida* plants. It appears that AnVY has a fairly restricted natural host range relative to its larger experimental host range.

This is the first report of a virus occurring in *A. lucida* L. and *A. genuflexa* Nutt. It is not clear if AnVY originated in Alaska and co-evolved with a particular plant species before infecting *Angelica* species or was recently introduced from symptomless invasive weeds or cultivated plant species such as parsley, celery, or carrot [5].

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